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Evidence for the Accelerated Degradation of Isoproturon in Soils

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Abstract: The herbicide isoproturon was degraded rapidly in a sandy loam soil under laboratory conditions (incubation temperature, 15°C; soil moisture potential, −33 kPa). Degradation was inhibited following treatment of the soil with the antibiotic chloramphenicol, but unaffected by treatment with cycloheximide, thus indicating an involvement of soil bacteria. Rapid degradation was not observed with other phenylurea herbicides, such as diuron, linuron, monuron or metoxuron incubated in the same soil under the same experimental conditions. Three successive applications of isoproturon to ten soils differing in their physicochemical properties and previous cropping history induced rapid degradation of the herbicide in most of them under laboratory conditions. There were, however, no apparent differences in ease of induction of rapid degradation between soils which had been treated with isoproturon for the last five years in the field and those with no pre-treatment history. A mixed bacterial culture able to degrade isoproturon in liquid culture was isolated from a soil in which the herbicide degraded rapidly.

Key words: isoproturon soils, enhanced biodegradation, bacterial culture, phenylureas

1 INTRODUCTION

Biodegradation is one of the most important processes controlling pesticide dissipation in the soil environment, and it can have an important influence on other aspects of behaviour in soil, such as movement to surface and ground waters, and effects on non-target organisms. However, where residual herbicides are concerned, they must persist in soil for a period after application in order to give an acceptable period of weed control.[1] Thus, the development of enhanced or accelerated biodegradation of herbicides in soil, defined as a microbial adaptation leading to rapid metabolism,[1–3] could have significant effects on both efficacy and environmental fate. The phenomenon of enhanced degradation of pesticides was first observed with the phenoxyalkanoic acid herbicides,[4,5] but more recently it has been reported to affect several other groups of compounds, including substituted ureas.[6–8]

Isoproturon (3-(4-isopropylphenyl)-1,1-dimethylurea) is a phenylurea herbicide widely used for pre- and post-emergence control of annual grasses and broad-leaved weeds in winter cereals.[9] Because of its widespread use and its properties of moderate persistence and relatively low adsorption, isoproturon has become an occasional water contaminant in some agricultural areas of the UK.[10] Degradation of isoproturon in soils has been reported to be primarily microbial in nature,[11–13] and is strongly dependent on soil temperature and moisture content.[12,14,15] Although isoproturon may often be applied to the same soil in the field in successive seasons, and its degradation is microbial in nature, there is no definitive evidence of accelerated biodegradation of this herbicide in soils.

As part of a research project looking at the long-term adsorption of isoproturon in soils, we located a soil in which the herbicide appeared to degrade unusually quickly. The objectives of the experiments reported in this paper were:

1. to study the behaviour of isoproturon in this unusual soil, with particular emphasis on the degradation

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rates of repeated applications of isoproturon under laboratory conditions;
2. to investigate the possibility of enhanced biodegradation occurring in a range of soils differing in their physicochemical properties and cropping history;
3. to investigate the stability of other phenylurea herbicides when incubated with the soil capable of rapid degradation of isoproturon; and
4. to isolate micro-organisms from soil with the ability to degrade isoproturon in liquid culture, and to examine their ability to degrade related phenylurea herbicides.

2 MATERIALS AND METHODS

2.1 Herbicides and soils

Isoproturon is a crystalline solid with m.p. 155–156°C, v.p. 0·0033 mPa (20°C) and water solubility 55 mg kg^{-1} (20°C).[9] The isoproturon used in this study was a commercial 500 g $litre^{-1}$ suspension concentrate formulation, plus pure analytical grade herbicide (British Greyhound, Birkenhead, UK). Diuron, monuron and metoxuron were used as commercial 800 g kg^{-1} wettable powder formulations, and linuron was a commercial 500 g kg^{-1} wettable powder. The structural formulae of the compounds are given in Fig. 1.

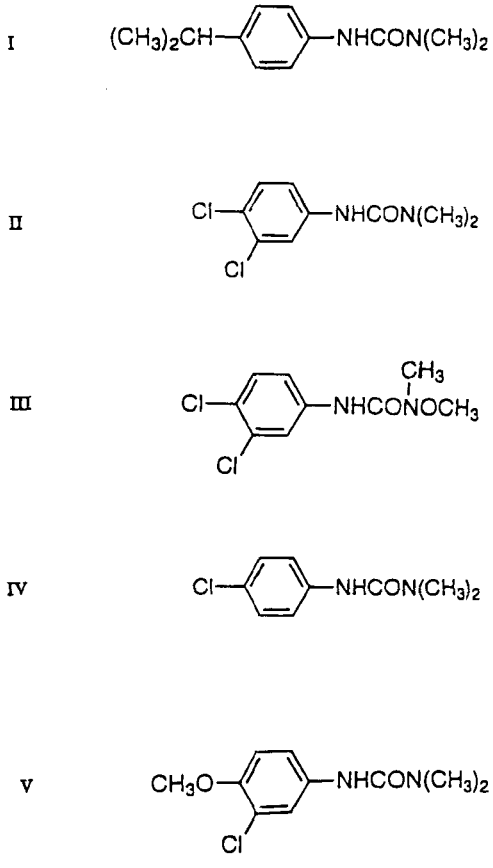

Fig. 1. Molecular structures of (I) isoproturon, (II) diuron, (III) linuron, (IV) monuron and (V) metoxuron.

Soil samples were collected from the top 10 cm of 10 different field sites located at Horticulture Research International, Wellesbourne. They were all classified as sandy loams or sandy clay loams containing 15–25% clay and over 60% sand. Other properties are listed in Table 1. The soil from one of these sites (Deep Slade) had been shown in other experiments to degrade isoproturon rapidly with total dissipation in six to seven days. Five of the soils (including that from Deep Slade) had not been treated in the field with isoproturon during the last five years and these are referred to as 'untreated' soils (U in Table 1), while the rest of the soils had been treated in the field in each of the last four years and these are referred to as 'treated' soils (T in Table 1). Samples were air-dried overnight, sieved to pass a 3-mm mesh and their moisture contents determined. To avoid cross-contamination between soils, separate sieves were used for the different soils, the

TABLE 1
Physicochemical Properties of the Soils, Moisture Contents at a Potential of −33 kPa and History of Treatments (T and U, Soils Treated and Untreated with Isoproturon in the Last Five Years)

Site	*pH*	*Organic matter* (%)	*Moisture content* (%) at *−33 kPa*	*Pretreatment*
Deep Slade	7·8	1·99	13·7	U
Pump Ground East	7·3	2·63	15·0	U
Sheep Pens	6·8	2·19	13·2	U
Townsend	5·2	2·64	14·0	U
Wharf Ground	7·0	2·66	13·9	U
Hunts Mill	6·5	2·14	13·0	T
Big Ground	6·3	2·32	16·3	T
Cottage Field	6·5	4·02	16·0	T
Plum Orchard	7·2	3·16	15·4	T
Pump Ground West	7·1	3·04	14·1	T

sieves were autoclaved before and after use, and aseptic techniques were used as far as possible throughout the experiments.

2.2 Isoproturon applications to soils and incubation studies

Two replicate amounts of each soil (1 kg) were treated with a suspension in water of formulated isoproturon to give a concentration of 10 mg AI kg^{-1} dry soil with soil moisture equivalent to a matric potential of −33 kPa (Table 1). After application of the herbicide, the soil samples were thoroughly mixed by passing at least four times through a sieve, and then three amounts of 300 g were transferred to loosely capped polypropylene containers (500 ml capacity; A, B and C for each replicate soil sample) which were incubated at 15°C. Moisture contents were maintained at a constant level throughout the experiment by adding distilled water as necessary.

The soil in all of the A replicates was sampled periodically over a period of 25 days and analysed for isoproturon by HPLC (see Section 2.5). After 25 days, the soils in replicates B and C were retreated with isoproturon and the B replicates sampled periodically during the next 25 days. After this period, replicates C were treated for the third time with isoproturon and again sampled periodically thereafter.

2.3 Incubation and microbiological studies with the Deep Slade soil

2.3.1 Influence of temperature, moisture content and addition of antibiotics on isoproturon degradation in soil

Duplicate subsamples (300 g) of soil from Deep Slade were treated with isoproturon (10 mg AI kg^{-1}) and incubated in polypropylene containers at temperatures of 5, 15 and 20°C with soil moisture at 13·7% (matric potential, −33 kPa). Further samples were incubated at 20°C only with soil moisture adjusted to 7 and 17% (matric potentials of −1500 and −5 kPa respectively). Additional samples of soil (30 g), in sterile conical flasks (100 ml), were treated with a solution of cycloheximide (2 ml, 750 mg $litre^{-1}$) or chloramphenicol (2 ml, 750 mg $litre^{-1}$) and incubated at 20°C for 24 h. There were 10 replicates for each antibiotic. After this time, an aqueous suspension of isoproturon was added to the soils to give a final concentration in the soil of 10 mg AI kg^{-1}. Flasks were thoroughly shaken, incubated at 20°C and sampled periodically by removing two flasks of each treatment for analysis.

2.3.2 Enrichment culture

The enrichment culture technique used to isolate bacteria from soil with the ability to degrade isoproturon was as described previously by Roberts *et al.*[8,16] A subsample (500 mg) of soil from Deep Slade which had been treated with isoproturon on three occasions (Section 2.2 above) was used to inoculate 20 ml of enrichment liquid medium. The medium contained no added carbon or nitrogen source other than analytical grade isoproturon. The herbicide was added to the medium as a solution in analytical reagent grade ethanol (10 g $litre^{-1}$) to give a final concentration of 25 mg $litre^{-1}$. The enrichment medium was contained in conical flasks (100 ml) which were shaken at 25°C. All flasks were sampled periodically and when the herbicide concentration had decreased to less than 50% of its initial value, flasks were subcultured by transfering 0·5 ml to new flasks containing 20 ml of liquid enrichment medium plus isoproturon. There were three inoculated replicates and three uninoculated controls for each of three incubation cycles.

2.4 Incubation studies with other phenylurea herbicides

Further subsamples (2 × 300 g) of soil from the Deep Slade site were treated with the commercial formulations of diuron, linuron, monuron and metoxuron as described above (Section 2.2). All samples were incubated at 15°C and with soil moisture at a matric potential of −33 kPa (13·7%). Incubation experiments with the 1,1-dimethyl substituted urea herbicides diuron, monuron and metoxuron were also made in Deep Slade soil which had been treated on two previous occasions with isoproturon in the laboratory.

The ability of the mixed microbial culture to degrade diuron, linuron, monuron and metoxuron was examined in conical flasks (100 ml) with mineral salts liquid medium (20 ml)[8,16] containing the respective analytical grade herbicide solution at a concentration of 25 mg $litre^{-1}$. The flasks were inoculated with subsamples (1 ml) of the liquid cultures at 50% isoproturon degradation as described above (Section 2.3.2). The flasks were incubated at 20°C and sampled periodically. There were three replicates of all treatments including uninoculated controls for each herbicide.

2.5 Soil extraction and herbicide analysis

The herbicides were extracted from soil (20 g) by shaking with HPLC grade methanol (20 ml) for 1 h on a wrist-action shaker. The samples were allowed to stand until the soil had settled, after which aliquots of the clear supernatants were analysed by HPLC without further treatment. The column used was Spherisorb ODS 2 (150 × 4·6 mm), the solvent system was methanol + acetonitrile (HPLC grade) + water (30 + 30 + 40 by volume) at a flow rate of 1 ml min^{-1}, and all compounds were determined by UV absorbance

at a wavelength of 240 nm. Herbicide concentration in liquid cultures was also determined by HPLC. In order to stop microbial degradation, subsamples for analysis (1 ml) were diluted with an equal volume of acetonitrile.

3 RESULTS AND DISCUSSION

3.1 Isoproturon incubation studies in soils

The dissipation patterns of isoproturon after the first, second and third application to the five 'untreated' and the five 'treated' soils are shown in Figs 2 and 3 respectively. The data are means of two replicates, with the exception of those for the third treatment of the soils from Hunts Mill and Big Ground (Fig. 3) where there was a large difference in residue decline patterns between the replicates. A particular feature of the data in Fig. 2 is the unusually rapid degradation of isoproturon in the soil from Deep Slade, even after the first treatment, when it was degraded completely within 10 days. Successive treatments degraded even more rapidly, and after treatment for the third time, isoproturon degraded completely in this soil in just five days. This potential to degrade isoproturon was totally unexpected considering the low organic matter content of

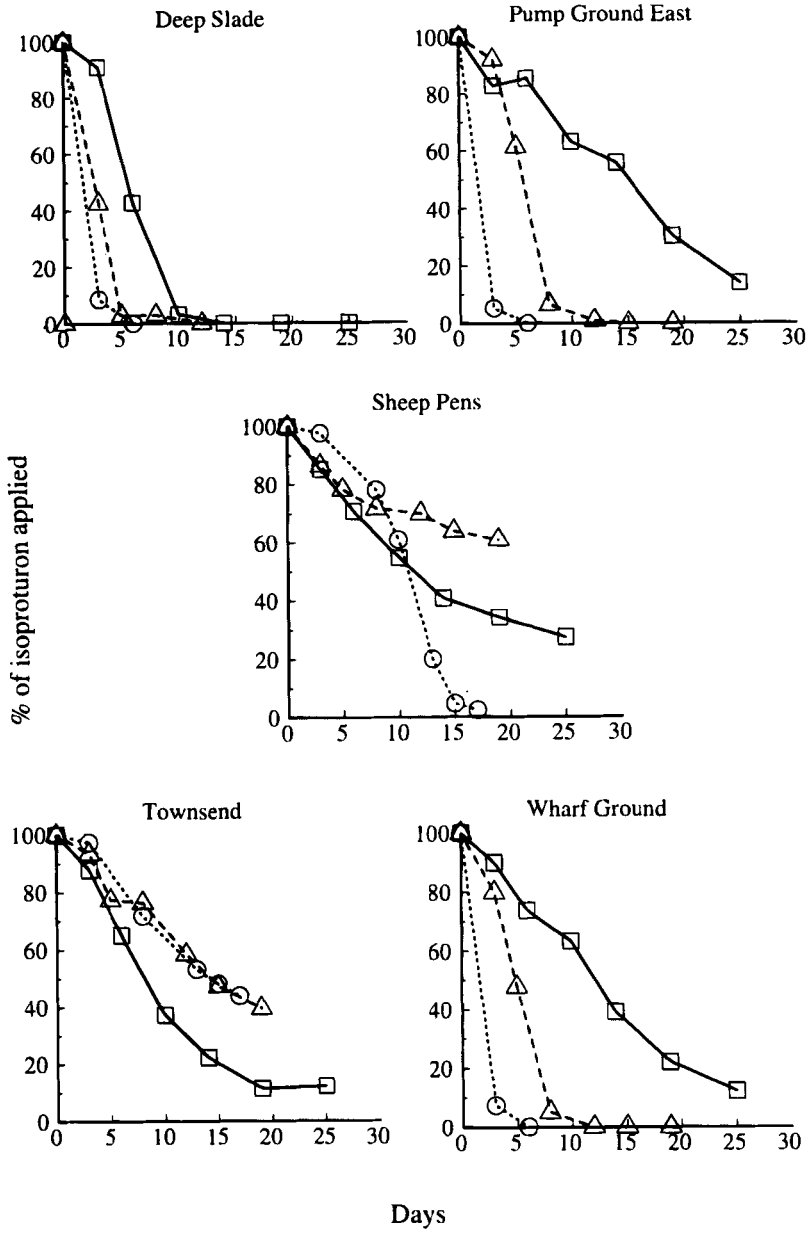

Fig. 2. Degradation of isoproturon in soils that had not been treated with isoproturon in the field during the previous five years. The results indicate the decline patterns at 15°C after (□) one, (△) two and (○) three successive treatments in the laboratory.

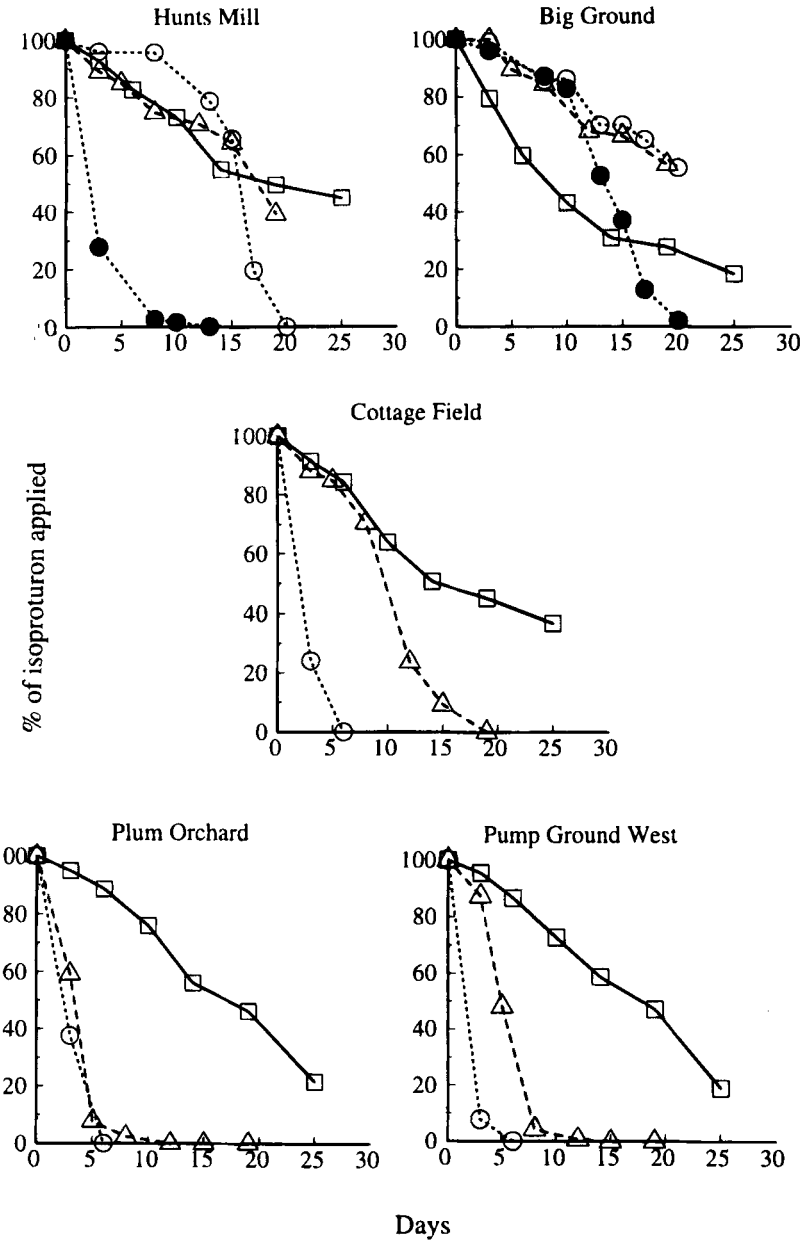

Fig. 3. Degradation of isoproturon in soils that had been treated with isoproturon in the field in all of the previous four years. The results indicate the decline patterns at 15°C after (□) one, (△) two and (○, ● for replicates 1 and 2) three successive treatments in the laboratory.

the soil (Table 1), which can be considered an indirect measurement of soil biological activity,[17] and the fact that this soil had not been treated with isoproturon in the last five years. The soil from Hunts Mill, a field adjacent to the Deep Slade site and of similar organic matter content, although lower pH (Table 1), did not exhibit the same behaviour (Fig. 3). The Hunts Mill soil had an extensive history of isoproturon application. The high pH of the soil from Deep Slade (7·8) could favour microbiological degradation of the herbicide, although further studies would be required to confirm this. Three of the five 'untreated' soils exhibited rapid degradation of isoproturon when treated for a second and third time with the herbicide (Fig. 2). In the soil from Sheep Pens, the second dose of isoproturon degraded more slowly than the first, and it was not until the third application that significant enhancement was observed. In the soil from the Townsend site, degradation proceeded more slowly when treated for both the second or third time. Slower degradation with time could be attributable to changes in the biological activity in stored soils.[18] It should be noted that the Townsend soil had the lowest pH of the soils examined, and this suggests that further experiments are required to examine the possible interaction between soil pH and the microbial degradation of isoproturon.

The results in Figs 2 and 3 indicate that induction of rapid degradation of isoproturon in laboratory incubations is independent of field pretreatment history, which has been shown in previous experiments to be an important factor controlling the degradation rates of other herbicides[6,7,19] including phenylureas such as linuron.[6,7] This suggests that highly active populations of organisms able to degrade isoproturon are not established in field soils as a result of normal agricultural use of the herbicide. The unusual behaviour in the treatments involving soil from Hunts Mill and Big Ground (Fig. 3), where there was a large difference between replicates in the apparent lag period before rapid degradation began, indicates that low and variable populations of organisms able to degrade isoproturon may be present in these soils compared with those present in other soils from the experimental farm at HRI, Wellesbourne.

3.2 Incubation studies and microbiological studies in the Deep Slade soil

Following the observation that the soil from Deep Slade was able to degrade isoproturon very rapidly, further incubation experiments were made with this soil to examine the influence of varying temperature and moisture content on rates of loss. The results are shown in Fig. 4(a). Over the time period of these experiments, there was little degradation at 5°C in moist soil, or at 20°C in the soil at 7% moisture (matric potential, −1500 kPa). This agrees with results from previous studies of isoproturon degradation in soils[12,14,15] which indicated slower degradation of the herbicide at low temperatures and low soil moisture contents. Low temperature will reduce the activity of soil microorganisms, although there is evidence with the herbicide napropamide that enhanced degradation can be induced at temperatures as low as 5°C.[20] On the other hand, soil moisture content has been shown to be critical to expression of enhanced biodegradation,[1] since it affects not only the physiology of the pesticide-degrading microflora but also the desorption rate and availability of the pesticide, which will have a direct effect on degradation.[21]

The effect of addition of antibiotics is illustrated in Fig. 4(b). Treatment of the soil from Deep Slade with the antibacterial antibiotic chloramphenicol[22] inhibited degradation of isoproturon, whereas no significant effect was observed from the antifungal antibiotic cycloheximide.[22] These results confirm the microbiological nature of the accelerated degradation of isoproturon in this soil and indicate the involvement of soil bacteria.

Isoproturon degradation in shake flask culture in the mineral salts medium during repeated subculturing is shown in Fig. 5. When inoculated with 500 mg soil

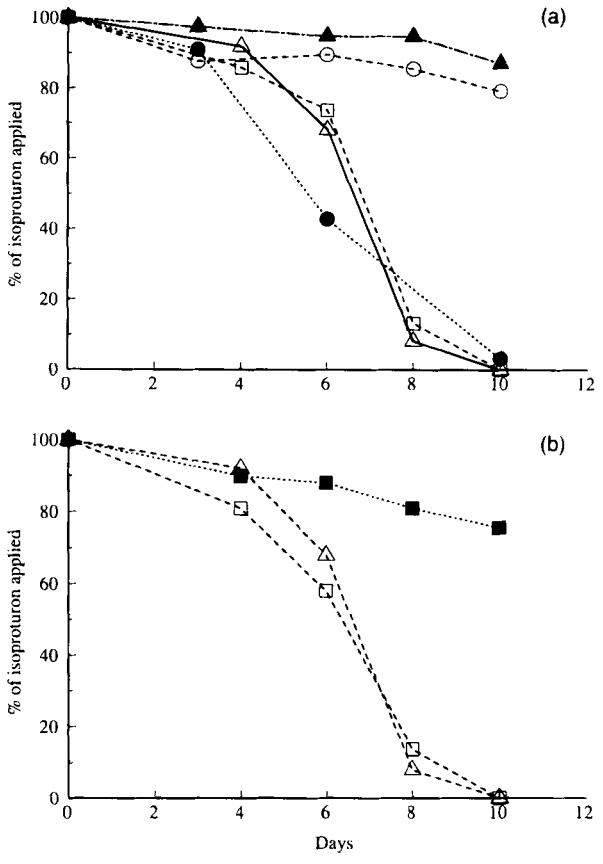

Fig. 4. Degradation of isoproturon in Deep Slade soil: (a) Incubation temperature 20°C with soil moisture at (○) 7%, (△) 13·7% and (□) 17% and with soil moisture at 13·7% with incubation temperatures of (▲) 5° and (●) 15°C; (b) Incubation temperature 20°C with soil moisture at 13·7% in soil (△) without antibiotics and treated with (■) chloramphenicol or (□) cycloheximide.

from Deep Slade (first cycle), there was rapid degradation of the herbicide in the liquid medium. This was also observed when 0·5 ml of culture was transferred in a second and third cycle, such that rapid degradation

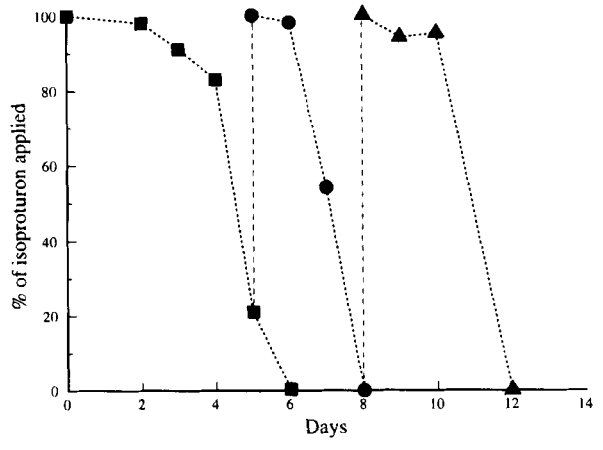

Fig. 5. Isoproturon degradation in shake flask culture in an enrichment medium during repeated subculturing using Deep Slade soil (pretreated twice with isoproturon) as inoculum. Vertical lines indicate times when the culture was transferred to fresh liquid enrichment medium.

was taking place eventually in the absence of soil and with isoproturon as the only carbon and nitrogen source. Incubation experiments with the mixed culture also indicated the presence of a main degradation product with the same retention time as monomethyl-isoproturon, which has also been identified as a main metabolite in soils.[13]

3.3 Incubation studies with other phenylurea herbicides

The patterns of residue decline of the herbicides diuron, linuron, monuron and metoxuron in soil from the Deep Slade site are compared with that of isoproturon in Fig. 6(a). The experiment lasted for 20 days at an incubation temperature of 15°C and with soil moisture at 13·7%. Rapid degradation was only observed with isoproturon, whereas in the case of the other phenylurea herbicides, particularly with the more stable compounds diuron, linuron and monuron, degradation was still very slow in this soil. Even with the relatively unstable compound metoxuron, with a reported half-life in soils of 10–30 days,[9] there was no evidence of unusually rapid degradation in the Deep Slade soil. Degradation of 1,1-dimethyl-substituted ureas generally involves demethylation,[23] and this has been confirmed to be the major degradation pathway of isoproturon in soils.[13] This was also shown to be a significant route for isoproturon degradation in the present study with soils and with the mixed bacterial culture, since the mono-methyl degradation product of isoproturon was the only metabolite identified (data not shown). The stability of the other 1,1-dimethyl-substituted ureas used in this study (diuron, monuron and metoxuron) indicates that, although degradation pathways might be the same, the micro-organisms involved in degradation may well be highly specific to a single compound. Similar results were observed with a mixed culture of organisms able to degrade linuron.[8] The culture showed limited ability to degrade other 1-methyl-1-methoxy-substituted ureas such as monolinuron and chlorbromuron but was unable to degrade metobromuron or any 1,1-dimethyl-substituted ureas. Fig. 6(b) shows the results of incubation experiments with the 1,1-dimethyl-substituted ureas diuron, monuron and metoxuron in soil from Deep Slade following pre-incubation of the soil on two occasions with isoproturon in an attempt to maximise its degradative potential. Metoxuron degraded rapidly with a half-life of about 15 days in both soil samples but there was no evidence for its accelerated degradation in the pre-treated soil. Both diuron and linuron were stable in the 'untreated' Deep Slade soil (Fig. 6(a)), but they showed an initial rapid loss followed by much slower loss in the pre-treated soils (Fig. 6(b)). This unusual pattern of behaviour could have resulted from the activities of extracellular enzymes which may have been present in the soil initially, but not produced in the absence of isoproturon. Clearly further work is needed to investigate this possibility.

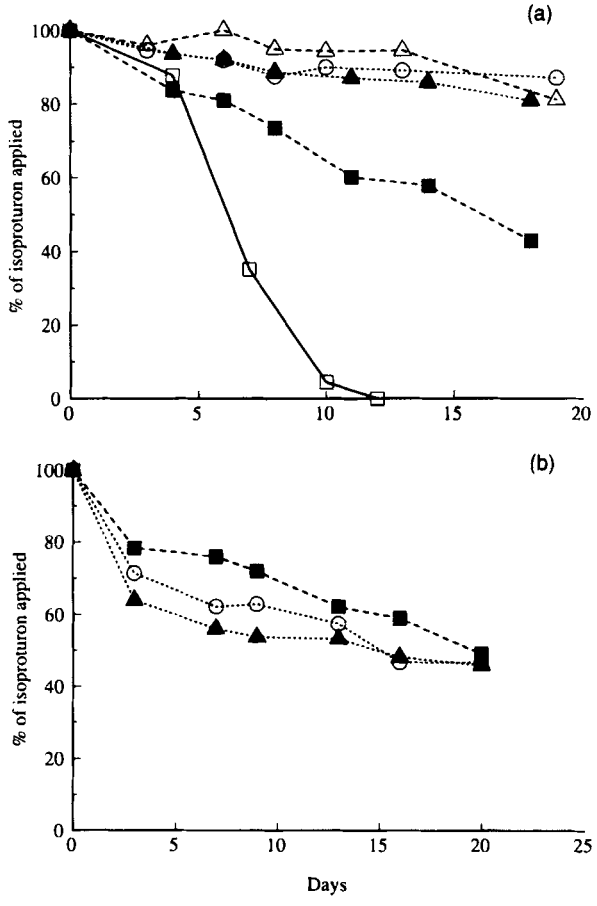

Fig. 6. (a) Degradation of the phenylurea herbicides (□) isoproturon, (▲) diuron, (△) linuron, (○) monuron and (■) metoxuron in the Deep Slade soil incubated at 15°C and 13·7% moisture content and (b) degradation of (▲) diuron, (○) monuron and (■) metoxuron in Deep Slade soil previously treated with isoproturon.

Incubation experiments in liquid medium with the mixed culture isolated from the Deep Slade soil (data not shown) also confirmed the high specificity of the micro-organisms for degradation of isoproturon. After an incubation period of about 20 days, bacteria able to degrade isoproturon showed no ability to utilise diuron, linuron, monuron or metoxuron as sole source of carbon and nitrogen.

4 CONCLUSIONS

A soil has been located with potential for unusually rapid biodegradation of the herbicide isoproturon under laboratory conditions. The results indicate that soil bacteria, possibly favoured by the high pH of the soil, are involved. This rapid degradation is apparently

highly specific to isoproturon, which suggests that it may result from stimulation of the activities of specific enzymes. The implications of this phenomenon are of particular interest considering the wide use of isoproturon. There is the possibility that the isolated microorganisms could be used for bioremediation of contaminated soil or water systems as suggested by Feakin *et al.*[24] Further experiments are required to determine the significance of enhanced degradation in the field, since there is little evidence for reduced activity of the herbicide following repeated application.

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